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14. ABSTRACT Anti-PSMA designer T cells are Autologous T cells that are gene-modified to express chimeric immunoglobulin T cell receptors (IgTCR) that recognize the prostate specific membrane antigen. We will conduct Phase I dose-escalation trial with infusion of designer T cells into prostate cancer patients after non-myeloablative (NMA) conditioning. This procedure will allow for the stable engraftment and persistence of the infused cells for improved therapeutic effect. The first year efforts have been in preparation of a clinical-grade vector producer cell (VPC) line, production and testing of clinical vector supernatants, and in harmonizing IRB-recommended protocol modifications and completing administrative features in advance of the trial. In year 2, the vector was received from NGVL after production delay. Patients will commence enrollment in year 3, January 2008.					
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ANNUAL REPORT

Introduction

The study is a clinical trial of anti-PSMA designer T cells in prostate cancer. The second year under the study has been focused on two major tasks: 1. finalizing vector cloning and selection, and vector production in collaboration with the National Gene Vector Lab (NGVL); and 2. completing administrative tasks in preparation for the clinical trial.

Body

Tasks from Statement of Work.

Task #1. Vector production/delivery (completed)

a. Cloning optimal vector producer cell (VPC) (completed)

Testing for the best MFG-S3D8V5 retroviral supernatant from PG 13 transduced clones produced at the National Gene Vector Laboratory (NGVL) for the purpose of preparing our prostate gene therapy GMP supernatant. We provided purified plasmid DNA for the MFG vector to the NGVL from which they would create a new VPC under GMP conditions. This work to produce the VPC was done at the NGVL. In turn, the BDL conducts the tests of the titers to determine the best clones.

To find the best cells to use for this screening, we tested three cell types to determine which gives the best transduction rate. Jurkats (a human CD4 T cell lymphoblast cell line), 293 (human embryonic kidney cell line), and PBMCs (peripheral blood mononuclear cells, which is our source for fresh T cells) from buffy coats were tested. These cells were transduced two times with either IgTCR or Tandem vectors (two other test vectors in the lab). The best cell type of the three was the 293 cells which gave 69% transduction for the IgTCR and 44% for Tandem post 48-hr gene expression. The Jurkats gave 5% IgTCR and 7% Tandem, and the PBMCs only 0.6% IgTCR and 0.45% Tandem. A graphical analysis showed that there was a constant ratio between 293 cells and the normal activated T cells, but that titer was more sensitive with the 293 cells. Therefore, we did initial screening with the 293 cells and then confirmed relative titers in normal activated T cells, the cells that would be used for the clinical trial.

Testing 1st (38 clones) and 2nd (21 clones) batches of clones; retesting selected clones (12); final testing/selecting the best clone. The supernatants (1:3 dilutions) from 59 clones from NGVL were used to transduce activated T cells twice. Facs analyses were done; the 3 best clones (clones 1, 6 and 10) were retested in both 293 and activated T cells. Gene transfer rates for the 293 and the T cells after 3-day post gene expression were compared for the three clones. Clone 6 gave 66.1% in the 293 cells and 11.2% in the T cells compared with 55.3% (clone 1) and 49.2% (clone 10) in 293 and 10.2% (clone 1) and 6.8% (clone 10) in activated T cells. Clone 6 was selected as the best for manufacturing the GMP-grade supernatant for prostate cancer clinical trials.

Associated studies to improve gene therapy procedure:

1. As an adjunct study to support the trial, we attempted to validate a replication competent retrovirus (RCR) Serologic Assay using anti-CD46 antibody for detecting GALV envelope protein. The GALV envelope is reported to cross-react with CD46. This assay is based on antibody detection in the serum of patients. If the anti-viral antibody is detected by GALV positive cells, that means the patient has retrovirus growing inside his body.

Results: Serial dilutions of the antibody which putatively recognizes the retroviral envelope (GALV) were made over a range of 10 to 0.01 *ug/ml* sensitivity with NIH3T3 cells expressing GALV env. The antibody was not detected in this assay. It was concluded after two tests that this antibody does not work in this application as a positive control with which to validate the test. Therefore, it does not meet the FDA criterion for a controlled assay. Accordingly, the more expensive and prolonged culture assay for RCR will be continued for this study.

2. We also examined more efficient means for transduction to bring up the fraction of modified cells in the designer T cell preps. In a titer/dilution assay, we discovered an unexpected result with increased titer with increasing dilution. We determined infectious titer of dilutions of retroviral supernatant: 1, 1:3, 1:9, and 1:27 in Jurkats and 293 cells transduced with the best viral supernatant harvests from PG13/Tandem sorts 0-3. PG13/Tandem is a heterologous vector used for control tests.

Results: The gene transfer rate and the fluorescence intensity for all sorts was highest at the 1:3 dilution, sort 3 was slightly higher for the 1:9 dilution. This result may reflect an increase in nutrition for the cells thereby enhancing their infection. We may consider diluting the *s/n* 1:3 in future experiments. This test was repeated with similar results: in 3 out of 4 tests, the 1:3 dilution gave the highest gene transfer rate.

b. Production of clinical quantities of vector (completed)

The selected VPC clone (#6) was indicated to NGVL. The cells were expanded at NGVL and the master cell bank (MCB) and safety testing were completed. The vector production and safety testing were completed. The vector was delayed due to production backlog. Vector was received June 2007.

Task #2. Study preparations

a. Laboratory standardizations (completed)

The detection of designer T cells in the tumor after patient biopsies will be by staining with antibody that recognizes the V5 epitope that was inserted into the IgTCR extracellular domain. Control validation tests were with prostate cancer tissue that was injected with a dilute solution of designer T cells and then processed as fixed or frozen tissue with immunohistochemistry (IHC). These tests were performed with the Pathologist (M Stancu, MD) and the lab protocol manager (Q Ma, PhD) and one of the clinical leaders on the study (P Somasundar, MD). This was completed, showing designer T cell detection in fixed or frozen tissue.

b. Staff training/orientation

Robin Davies, RN, research nurse, created Case Report Forms (CRF) for the Study that were submitted with the original application. These forms have been updated during the last year. A further draft version of the CRF has been prepared by Ms. Davies and Katarzyna Szulc, BS, data manager, so that more specific information can be collected. This is the primary training document for the medical staff who will be administering the T cells and managing the patients. The CRF was finalized in 8/07.

In December 2007, an in-service will be conducted on the BMT unit for staff orientation in advance of initiating patient treatments.

Task #3. Clinical protocol implementation

a. Protocol modifications

1. Prior study modifications

Principal Investigator Richard P Junghans, PhD, MD and Nurse Coordinator Robin Davies, RN attended the US Army Grants Award Meeting on May 4-5, 2005, where the outline of interactions between the grantees and the DOD was presented, including the compliance with the human subjects review of the DOD. This DOD meeting is used to mark the beginning of the past year's protocol activities. The clinical protocol (and consent) has undergone a number of revisions over the year to harmonize requirements between the DOD and RWMC IRBs. The history prior to the award is included to provide the full context of the activities undertaken in the period under the grant.

This study was initially submitted to the Roger Williams Hospital Human Research and Review Committee (IRB) on November 22, 2004. It was reviewed at a Full Committee meeting on December 21, 2004. Modifications were requested to the Protocol and Consent Form versions December 9, 2004. These modifications included a request to address the rationale for doses of chemotherapy used in the study. The response to these requests was submitted on December 28, 2004. The IRB then requested further information and changes in their second response dated January 14, 2005.

A second response to the IRB by the Principal Investigator was submitted on February 7, 2005. This included Protocol and Consent Form versions February 7, 2005. These versions were approved in a letter dated March 30, 2005. The approval was for the three month period March 30, 2005 through June 2005.

On May 12, 2005 the three month report was submitted to the IRB with February 7, 2005 versions of the Protocol and Consent Form. This was approved on May 26, 2005. Amendment #1 was submitted to the IRB on September 15, 2005 which included Cytosin dose changes. The Annual Review was then submitted on October 21, 2005. These were both reviewed on November 8, 2005. Requests for modifications were then made by the IRB. A response by the Principal Investigator was submitted on January 12, 2006. This response created Amendment #2 to the Protocol. These changes were accepted by the RWH IRB and signed on January 23, 2006 to approve versions January 12, 2006 Consent Form and February 7, 2005 of the Protocol.

During this period of time the Principal Investigator had submitted an application for funding to the US Army. A working version of the Protocol and Consent form were submitted in the form of Protocol and Consent Form versions March 28, 2005. This version was reviewed at the US Army Human Subject Research and Review Board (HSRRB) on September 14, 2005. As a result of this review, recommendations for modifications to both the Consent Form and Protocol were made. This request included 14 changes to the Consent Form, 14 changes to the Protocol and 11 additional items needing response and/or action. These changes were implemented per the changes requested by HSRRB, and resulted in versions October 15, 2005 of the Protocol and Consent Form.

On May 17, 2006 a request for final Roger Williams Hospital IRB approval of the Protocol and Consent Form was received from HSRRB. Since the Roger Williams Hospital had previously approved only RWH versions February 7, 2005 of the Protocol and version January 12, 2006 of the Consent Form, it was decided to submit the now HSRRB reviewed/approved (October 15, 2005 versions) of the Protocol and Consent Form to the RWH IRB. These were submitted in the form of Protocol (Amendment #3) version February 14, 2006 and Consent Form version February 14, 2006. A letter conditionally approving both Protocol and Consent Form was received on April 3, 2006, from RWH IRB. Modifications were requested to the Consent only resulting in version June 15, 2006. The modifications were approved on June 20, 2006. An Annual Review was submitted to the RWH IRB on September 30, 2006, which included Protocol version February 14, 2006 and Consent Form June 15, 2006. It was reviewed and approved on November 7, 2006.

Institutional Biosafety Committee approval was granted on April 7, 2005. The Data Safety Monitoring Board review took place on November 30, 2005. The Protocol was reviewed again by the IBC on December 20, 2006

due to the new versions created since the original IBC review. This review did not result in any new recommendations, since changes were mainly administrative, regulatory.

2. Study modifications in current award period

In 2007, we made a modification to the protocol consent form to incorporate the possibility of brain toxicity. Two reports appeared in 2006 and 2007 stating PSMA protein detection on Type II astrocytes in the brain [1,2]. A previous review from Memorial Sloan-Kettering Pathology Department stated there was no such expression in the brain [3]. Our own screening tests for the 3D8 antibody were negative for staining brain tissue, performed by University of California, Irvine, Department of Pathology under contract. Because of concern that this could present an unanticipated toxicity, we did additional studies to look for such staining. There does appear to be some low level staining on some astrocytes.

The effect of targeting a positive fraction of astrocytes is unknown. Astrocytes are postulated to maintain the blood-brain barrier and to have nutritional roles for neurons [4], but no disease of astrocytes exist from which to infer with confidence the potential effect of such attack. Thus, we are uncertain on two levels: 1. if any astrocytes will be attacked, and 2. how patients will be affected if astrocytes are attacked. Accordingly, we modified the consent form to inform patients of this uncertainty.

This study has been submitted for Annual IRB renewal in November 2007. No patients have been entered at this time.

b. Patient enrollment

The vector was received from the National Gene Vector Laboratory (NGVL) and is on site at RWH. All safety testing has been completed. Staff are currently reviewing procedural and clinical logistics for the enrollment and treatment of subjects, planned for January 2008.

We elected to defer initiation of the prostate study until after completing the first dose level of the anti-CEA designer T cell study. The reasons are as follows. The anti-CEA study and PSMA study have elements in common, but the PSMA study has additional elements that make the study more complex (Table 1). When we safely compete the 1st dose level with CEA, then elements 1-4, including all manufacturing steps, will have been safely validated. Then, if there are serious toxicities with the PSMA study, the unique elements of the PSMA study (5-7) will appropriately draw our attention, while permitting the CEA trial still to move forward. On the other hand, serious toxicities on the PSMA study prior to CEA initiation would bring scrutiny to all elements (1-7), potentially stymieing both the CEA and the PSMA study. The first patient on the CEA study was treated on November 26, 2007. The second is scheduled to enroll December 4, 2007. We project to be ready to enroll prostate cancer patients beginning in January 2008.

Table 1.

	CEA Tandem	PSMA
1. Vector Manufacturing	X	X
2. Designer T cell manufacturing	X	X
3. T cell infusion	X	X
4. Expected toxicities acceptable	X	X
5. Unknown toxicities possibly dangerous		X
6. High dose chemotherapy		X
7. IL2 co-administration		X

c. Laboratory studies

None to report at this time.

Task #4. Data management

The Case Report Form (CRF) has been finalized. A Study Calendar was created for Adverse Event reports and Annual reports, with contact information for all involved agencies.

Key Research Accomplishments

None to report at this time.

Reportable Outcomes

Journal publications

Methods for optimal vector producer cell (VPC) generation:

EL Beaudoin and RP Junghans. Sorting vector producer cells for high transgene expression increase retroviral titer. J Virol Methods; 2007, accepted pending revisions.

Conference oral presentations

Presentation at Manchester, UK, December 2006

“Phase I clinical trial and U.S. regulatory experience with anti-CEA designer T cells in adenocarcinoma”

American Association of Immunologists meeting (Miami, Florida; 05/18/2007-05/22/2007)

“Phase I clinical and U.S. regulatory experience with designer T cells in cancer”

Conference poster presentations

Department of Defense Prostate Cancer Research Program – IMPaCT Meeting (Atlanta, Georgia; 09/05/2007-09/08/2007)

“Phase I trial of anti-PSMA designer T cells in advanced prostate cancer”

Conclusions

The study has moved forward in the vector preparations and in the study administration. It is anticipated that enrollments will begin in January 2008.

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Appendices

None.